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PYRIDOSTIGMINE AND WARM WATER DIVING PROTOCOL 90-05: I. PROTOCOL AND GENERAL RESULTS

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TECHNICAL REVIEW AND APPROVAL
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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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A study was conducted to determine whether pre-treatment with pyridostigmine affected performance of divers in warm water. This report contains a review of pyridostigmine effects, experimental design, and general results obtained from the study. Detailed results for thermal, cognitive performance, physical performance, and hydration status can be found in accompanying NMRI Technical Reports listed in Table 1. Ten U.S. Navy divers performed two 7-hour warm air/water test exposures. Each test consisted of a 4-hour pre-dive exposure to 37.8 ° C (100 ° F) air at the surface, followed by a 3-hour dive in 34.4 ° C (94 ° F) water at a depth of 20 fsw (1.6 ATA) breathing 100% O_2. Subjects performed light-moderate leg exercise during the immersion phase, which was equivalent to swimming at 0.6-1.1 knots. During the first 2 h of immersion subjects exercised in a pattern of 30 min work (V_{O_2} = 1.0 ℓ/min) and 10 min rest. During the last hour subjects exercised in a pattern of 5 min

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light work, 10 min moderate work (V_{O_2} = 2.0 ℓ/min), and 5 min rest. Tests were conducted in a balanced order: once after the subjects ingested pyridostigmine (one 30 mg tablet every 8 h) for 2 days prior to testing and once after ingesting placebo tablets over the same time period. The interval between tests was 5 days for each diver. Prior to the first exposure subjects were acclimated to heat stress by spending 90 min per day for 5 days in 100 ° F air. Heat acclimation was continued on alternate days in the period between tests. Subjects ate MRE rations for 2 days prior to each test and wore camouflage utilities and footwear throughout each test. Thermal balance during dry and wet phases was obtained from regional skin temperatures, rectal temperature, and regional heat fluxes. Cognitive performance was assessed by the NMRI Psychological Assessment Battery (PAB) administered prior to the start of each exposure, at the 2nd and 4th hour of the dry phase, and after completion of the dive phase. Motor coordination in the dry and wet phases was tested by the time it took to assemble 12 nuts and washers on a bolt. Visual acuity was measured while ambient lighting was decreased. Grip strength was measured in the dry phase. Heart rate, ventilation, and oxygen consumption were measured during the dry and wet phases. Hydration status was assessed by pre-exposure to post-exposure changes in body weight, changes in plasma volume, and in urine production. Pyridostigmine lowered heart rate by 7 beats/min, and decreased mean skin temperature 0.1-0.2 ° C, during the dry phase. No significant effects of pyridostigmine were noted that would limit a diver's performance in warm water. The magnitude of the thermal stress alone was sufficient to decrease cognitive performance by 20-40%. Average rectal temperature at the end of the 7-hour exposure was 38 ° C, indicating that hyperthermia was unlikely to limit dives requiring light to moderate work. A third test exposure, identical to the first two except no drug was given and divers breathed air at 20 fsw, revealed no meaningful differences between air and O_2 breathing on performance.

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1. INTRODUCTION

Military personnel may, at times, be issued pyridostigmine bromide tablets as a prophylactic measure against organophosphate nerve agents. Studies examining the effects of pretreatment with pyridostigmine on heat tolerance, cognitive function, and exercise capacity have been conducted under dry, but not immersed, conditions. The drug has cardiovascular, neuromuscular, and thermoregulatory effects that could limit the performance of a diver. Warm water diving poses a thermal challenge that, by itself, may limit performance. The combination of pyridostigmine and warm water diving may have additive or potentiating effects that would result in a greater decrement in mission performance than either perturbation alone. A third factor, breathing gas, may also alter the diver's work tolerance, even in the absence of drug or thermal stress. Narcotic effects of breathing nitrogen mixtures or the toxic effects of high oxygen mixtures are well-described for water temperatures ranging from 0-31 ° C at depths from 20-285 fsw. Little is known of these gas effects in warmer water.

The Bureau of Medicine (BUMED) and the Naval Medical Research and Development Command (NMRDC) tasked NMRI to develop guidance concerning the use of pyridostigmine pretreatment in warm water diving. A review of the pertinent literature on pyridostigmine revealed studies that were conducted in dry heat, but not warm water. Therefore, a preliminary research study was needed to determine whether the drug would produce notable changes in tolerance to heat, physical performance, or cognitive function in a hyperbaric environment.

The study was conducted from 14 September to 4 October 1990. General findings and recommendations were sent to operational commands via a message. A summary noting no adverse effects of pyridostigmine was also provided to OOC-32 for an AIG239 Diving Advisory.

The purpose of this report is twofold. First, to provide a brief review of the known effects of pyridostigmine bromide, especially in the low dose range (30 mg) used for pretreatment and with respect to its effects on heat tolerance. The second purpose is to present the details of how the research study was conducted, along with general results. Detailed results for thermal, cognitive performance, physical performance, and hydration status can be found in the four NMRI Technical Reports listed in Table 1.

2. REVIEW OF PYRIDOSTIGMINE EFFECTS

Chemical warfare "nerve gas" agents are usually organophosphate compounds that act by irreversibly inhibiting acetylcholinesterase. This enzyme is essential for degrading acetylcholine, the neurotransmitter at the neuromuscular junction that initiates muscle contraction. The parasympathetic nervous system contains acetylcholine with effects that slow the heart rate, constrict the pupil, increase gut motility, and increase secretions from bronchi, salivary, and sweat glands. In the central nervous system, depletion or pharmacologic blockade of acetylcholine produces memory loss and delirium,[1] whereas excess produces seizures.[2,3] Low doses of organophosphates lead to increased levels of acetylcholine, and thereby to nausea, vomiting, diarrhea, increased sweating, and reduced heart rate. Higher doses of organophosphate can produce limb and respiratory muscle

paralysis; and because these agents penetrate the brain, seizures and delirium can occur. The primary antidote for organophosphate poisoning is atropine; but this drug cannot be used prophylactically because it is likely to produce delirium and tachycardia.

Carbamates are agents that reversibly bind to acetylcholinesterase, forming an intermediate that dissociates slowly over a short period of time, leaving the enzyme activity subsequently intact. If a portion of the acetylcholinesterase is bound by these carbamates before exposure to organophosphates occurs, then this portion will soon be reactivated, and chances of survival after exposure will increase. The prophylactic administration of carbamates to animals exposed to organophosphates has clearly been shown to result in enhanced survival.[4,5] Pyridostigmine is a carbamate issued as 30 mg tablets to troops stationed in areas where there is a high risk of chemical attack.[6] One tablet is taken every 8 h when the risk of attack is probable. This dosage results in a 40% reduction in red cell acetylcholinesterase,[7] and plasma pyridostigmine levels of 10-15 ng/ml.[8,9]

The half-life of pyridostigmine in plasma after IV injection is 90 min.[10] The bioavailability of the drug after oral administration is much less: peak levels are obtained within 1.5-2.5 h, followed by a slow decline, with detectable levels present up to 6 or 8 h after a single dose.[10,11] Acetylcholinesterase inhibition increases gut motility that, in turn, retards absorption and prolongs the effect of orally administered pyridostigmine.[12] Frank malabsorption is known.[13]

Effects of pyridostigmine on exercise tolerance in the heat have been studied in dry conditions. Two human studies (9 subjects total) have suggested that skin blood flow

decreases after pyridostigmine ingestion, leading to reduced heat loss in subjects exercising at a moderate rate (9- to 10-minute per mile run for 30 min).[16,17] Average core temperature was higher by 0.1 ° C after exercise in 36 ° C heat (97 ° F), but did not rise during exercise at two lower temperatures.[17] Pyridostigmine did not alter performance or endurance during light exercise (walking) in men wearing chemical warfare protective garments in the "open", ready, configuration.[18] Monkeys studied at rest in 35 ° C (95 ° F) air had no change in core temperatures after pyridostigmine administration compared to monkeys taking no drugs.[19] The pyridostigmine-treated monkeys lost more fluid as sweat, however.

The studies in humans and primates have used light to moderate work loads. In rats run to exhaustion, pyridostigmine treatment reduced endurance and led to an increased rate of rise of rectal temperature.[20] Exercise under these conditions also increased blood urea nitrogen, creatinine, potassium, and lactate dehydrogenase. Pyridostigmine induced a mild hyperglycemia prior to exercise.[20,21] The doses of pyridostigmine used in these studies produced a 25-60% inhibition of circulating cholinesterase. The effects of pyridostigmine in this model could be reversed by the combined administration of atropine and diazepam.[22]

Pyridostigmine, unlike chemical warfare agents and insecticides, is minimally soluble in fats and therefore does not pass the intact blood-brain barrier at low doses. Much higher doses (equivalent to 2.1 grams oral dosage) can penetrate and cause seizures or other evidence of central nervous system activation.[23] Pyridostigmine, at doses used for chemical warfare prophylaxis, has been shown to cause only minimal impairment of

mental performance. Aircrew navigation and flight simulator performance tasks were unimpaired after several days of pyridostigmine administration (30 mg every 8 h).[24,25] Chronic administration also had insignificant effects on a variety of psychologic performance measures in human volunteers, although there was some effect on performance of simultaneous tasks.[26] Monkeys given pyridostigmine (30 mg every 8 h) exhibited no effects on oculomotor or visual function, although very high single doses (30 times the chemical warfare prophylactic dose) impaired visual tracking.[27,28] Central visual processing in cats is unaffected following pyridostigmine prophylaxis.[29] Contrast sensitivity is slightly reduced in dim light,[30] due to a slight reduction in pupillary diameter induced by acetylcholine excess. This is not an effect on central visual pathways.

Two studies assessed the possibility that pyridostigmine coupled with a physiologic stress might produce unwanted effects. Pyridostigmine did not worsen the physiologic effects of acceleration stress on tremor, mental performance or hand grip strength.[31] The combination of pyridostigmine and exercise in the heat did not change mental performance compared to that of exercise alone, but did worsen physical performance.[32] Severe heat stress (heat stroke) will damage the blood-brain barrier; enabling pyridostigmine to penetrate and potentiate seizures and mental status changes.[33] Studies with physostigmine, an acetylcholinesterase inhibitor that penetrates the brain, at doses equivalent to those of pyridostigmine, suggest that any mental performance changes due to carbamates are mild,[34,35] and would not significantly impede management of heat stroke.

As noted above, centrally acting cholinesterase inhibitors and cholinergic agonists may cause epileptic seizures.[2,3,36] Pyridostigmine, in doses 70 times those used for chemical warfare prophylaxis, is more potent than neostigmine in producing seizures.[23] Seizures are also a major sign of hyperbaric oxygen toxicity. The possibility that low-dose pyridostigmine might lower the hyperbaric oxygen seizure threshold has not been directly investigated. One study showed that hyperbaric oxygen was ineffective as a treatment for soman or pyridostigmine poisoning in rats, but did not increase the incidence of seizures.[37] Another possible way in which acetylcholinesterase inhibition might increase the risk of cerebral oxygen toxicity is by increasing cerebral blood flow, allowing more oxygen to reach the brain. Cholinergic antagonists reduce cerebral blood flow and agonists increase flow.[38,39] Pyridostigmine has not been directly tested for its ability to dilate cerebral arteries, but it is expected that an intact blood brain-barrier will prevent any response.

The side effects of pyridostigmine are mainly related to the effects of increased acetylcholine availability. Pyridostigmine is used extensively for the symptomatic treatment of myasthenia gravis; therefore, the side effects of doses up to 1500 mg per day (180 mg every 3 h) are well-known. The most frequently reported effects are those of increased gastrointestinal motility, i.e. diarrhea, bloating, and nausea. Bradycardia is unusual at therapeutic doses. Effects on blood pressure are the net result of decreased cardiac output and stimulation of the sympathetic ganglia, which make changes in blood pressure difficult to predict.[14,15] Bronchoconstriction also occurs. Cardiovascular and respiratory effects may lead to reduced exercise tolerance. Tremor and fasciculations

accompany high therapeutic doses of pyridostigmine. The tremor and fasciculations may reduce manual dexterity, and may also be mistaken for the early symptoms of hyperbaric oxygen toxicity. Mental performance can be affected by anticholinesterase agents that cross the blood-brain barrier, but pyridostigmine does not significantly penetrate the brain.

Chronic administration of acetylcholinesterase inhibitors produces a myopathy in experimental animals.[40,41,42] Patients taking large doses of pyridostigmine and other anticholinesterase agents become unresponsive to these drugs, and may develop a mild myopathy after years of use.[43] Chronic exposure to organophosphate pesticides is associated with complaints of muscle weakness, which usually resolve when the exposure is terminated.[14] The ultrastructural changes in the motor endplate in myasthenia gravis resemble those in experimental anticholinesterase inhibition.[40] This myopathy is produced by agents that release acetylcholine from the presynaptic terminal without affecting acetylcholinesterase.[44] The amount of damage is enhanced by exercise,[45] but muscle necrosis resolves quickly.[46] This form of myopathy can be completely prevented by blocking muscle calcium channels.[47,48] It is unlikely that this chronic effect of high doses of anticholinesterase inhibition would be seen with the low dose and short term administration contemplated for chemical warfare prophylaxis.

Other effects of high dose and long term anticholinesterase treatment should be mentioned. Stokes-Adams attacks (sudden loss of consciousness with ventricular standstill) were reported in a patient taking pyridostigmine and led subsequently to a fatal event.[49] The incidence of bradycardia and hypotension is low (12 of 1000

patients),[15] but such effects could increase the incidence of heat syncope in unacclimatized troops.[33] The bromide in pyridostigmine bromide can lead to spuriously elevated serum chloride levels.[50] There has been one case of frank bromide intoxication, with confusion and paranoia, in a woman taking 150 mg pyridostigmine every 3 h.[51] One case of parkinsonism, an obviously central nervous system effect, associated with a daily dosage of 360 mg pyridostigmine for 2 months, has been reported;[52] 360 mg pyridostigmine per day in one case, led to alopecia.[53] Finally, low doses of pyridostigmine may increase growth hormone secretion.[54] No short-term physiologic effects of growth hormone increase, other than glucose intolerance, are known.

3. RATIONALE FOR EXPERIMENTAL DESIGN

The present study was designed to provide a significant, yet practical, stress wherein any effects of pyridostigmine might interact with the environmental parameters to significantly reduce diver performance. A single profile was chosen for this preliminary investigation. Total exposure time for each test consisted of a 4-hour pre-dive exposure in the dry at an air temperature of 37.8 ° C (100 ° F); followed by a 3-hour dive to 20 fsw in 34.4 ° C (94 ° F) water. Divers breathed 100% oxygen during the dive phase. Light to moderate leg exercise was performed during the dive that was equivalent to swimming with fins at 0.6-1.1 knots.[57]

A pre-dive air temperature of 37.8 ° C was the upper limit that could be used in the hyperbaric chamber without risk of costly damage to the acrylic ports. A water temperature of 34.4 ° C was chosen because it is a reasonable upper limit that might be

encountered in ocean diving. It is also a temperature that, coupled with the planned exercise workload, would not likely cause a rapid onset of hyperthermia (core temperature > 39.5 ° C); thereby permitting sufficient time to evaluate drug effects on thermal stress.

The choice of 100% oxygen as the breathing gas was based on several factors. First, possible side effects of the drug include nausea, tremor, and visual disturbances; which are also possible toxic effects of hyperbaric oxygen. A 3-hour dive to 20 fsw is within U.S. Navy oxygen exposure limits, and the probability of oxygen toxicity is low. However, any additive effects of drug and O_2 might present symptoms that would otherwise be absent. Second, breathing 100% O_2 at deeper depths not only shortens the exposure time, but also increases the incidence of oxygen toxicity. Individual susceptibility to oxygen toxicity varies widely. Thus, it would be more difficult at depths > 20 fsw to determine whether the appearance of any signs and symptoms was due to random chance or drug effect. Lastly, it was reasoned that interactions of the drug and inert gas narcosis would be unlikely since the drug does not cross the blood-brain barrier. Helium-oxygen mixtures are recommended for deep dives. There was no reason to suspect that the drug would interfere with HeO_2 decompression schedules. Furthermore, deep dives usually involve short bottom times which, in turn, would compromise the amount of time necessary to assess drug effects on fatigue or thermal balance. Therefore, breathing 100% oxygen represented the most challenging dive profile, where any adverse effect of pyridostigmine might be observed.

Test trials were also done while breathing air at 20 fsw in order to determine whether thermal balance was different than when breathing 100% O_2. These trials were done in the absence of pyridostigmine pretreatment. Thus, the air trials enabled a further dissection of drug and thermal effects by cross-correlation analysis with air vs O_2 effects.

4. EXPERIMENTAL PROTOCOL

a. Subjects: Ten U.S. Navy divers volunteered to participate in the study. Their physical characteristics are presented in Table 2. An additional two divers agreed to perform the warm water dives in the absence of drug in order to verify instrumentation, procedures, and the magnitude of the thermal stress imposed by the experimental protocol. The study was approved by the NMRI Committee for Protection of Human Subjects, and all subjects signed informed consent after a full briefing on the nature of the study.

b. Exposure Profile: All tests were conducted in the Man-Rated Chamber Complex in the Diving Medicine Department. Two subjects were tested simultaneously. Each test began with a 4-hour pre-dive exposure at the surface in a dry chamber, with an air temperature of 37.8 ° C (100 ° F), and a relative humidity of 50%. Subjects were seated at rest for most of this period, except when performing one of the tests described in Section 5. During the dry phase subjects drank water at a rate of 1 ℓ per hour.

Immediately following the dry phase the two subjects entered the wet pot chamber. They donned a full face mask supplying gas through a demand regulator and entered the

wet pot, whose water temperature was 34.4 ° C (94 ° F). Subjects were seated in a semi-recumbent position on a cycle ergometer. Their heads were approximately 2-4 inches below the surface. The chamber was compressed with air to 20 fsw (1.6 ATA). During the first two hours of the dive, subjects exercised at a light workload in a pattern of 30 min of work and 10 min of rest. The light workload (bike setting of 25 W) was chosen to produce an oxygen consumption of about 1.0 ℓ/min, which equates to swimming with fins at a speed of 0.6 knots. During the 3rd hour of the dive, subjects exercised in a pattern of 5 min at the light workload, 10 min at a moderate workload, and 5 min of rest. The moderate workload (bike setting equal to 1.0 W/kg body weight) was chosen to produce an oxygen consumption of about 2.0 ℓ/min, which equates to swimming at 1.0-1.1 knots. Following the last rest period, the chamber was decompressed to the surface and post-dive testing was instituted.

Immediately post-dive the subjects sat in a chair to have their heart rate and blood pressure recorded, then stood for 5 min to assess the magnitude of orthostatic changes in rate and pressure. Subjects then moved back into the dry chamber in order to obtain a venous blood sample and perform a post-immersion cognitive test.

Each of the 10 subjects was scheduled to be tested on 3 occasions. The dry phase of the test was the same on all occasions. All 10 subjects performed the first 2 tests in a balanced order to study the effect of pyridostigmine. During these tests, subjects breathed 100% oxygen at depth. A period of 5 days elapsed between tests for each pair of subjects. The third test (6 of 10 subjects + the 2 additional divers) was conducted 8 days after completing the second test; in the absence of drug and breathing air at 20 fsw.

This latter test was conducted to determine if there was a significant difference between air and oxygen on the magnitude of the thermal stress.

c. Pre-Test Work-ups: Subject body composition and maximum aerobic capacity were measured 5-10 days prior to the first experimental exposure. The time constraint to complete this protocol precluded an extensive heat acclimatization schedule. Nonetheless, subjects were acclimated to 100 ° F air for 5 consecutive days prior to their first test exposure, and on alternate days between tests. The acclimation occurred in an environmental chamber located in the Environmental Medicine Department. Acclimation exposures consisted of 90 min per day of three bouts of 25 min leg exercise and 5 min rest. The bicycle workload was 1.0-1.5 W/kg. Water was ingested at a rate of 1 ℓ/h during acclimation periods.

Diet was standardized among subjects by having them eat MRE (Meal Ready to Eat) rations for 2 days prior to each test. Subjects also ate a MRE breakfast on the morning of each test, approximately 2 h prior to the start of the dry phase. Alcohol and nicotine were restricted for 2 days prior to each test. Caffeine consumption was limited to the equivalent of 3 cups of coffee per day for 2 days before testing, but no caffeine was consumed for 8 h prior to the start of the dry phase.

d. Pyridostigmine Dosage: Drug trials were conducted in a balanced order; with 5 subjects undergoing the first test after pretreatment with pyridostigmine and 5 subjects tested first after ingesting a placebo. Pyridostigmine tablets (30 mg each) were obtained from standard issue blister packs (NATT, NSN 6505-01-178-7903). One tablet was ingested every 8 h for 2 days prior to the test at 0600, 1400, and 2200 h. A final dose

was administered at 0600 on the day of the test, approximately 2 h prior to the start of the dry phase. Placebo trials were conducted after the subjects ingested saccharine tablets in the same temporal sequence as drug trials. Saccharine tablets have about the same color, size, and consistency as the pyridostigmine tablets. Subjects, and all but one investigator, were blinded with respect to drug/placebo trials. Drug or placebo tablets were ingested with 200-300 ml of water.

e. Subject Instrumentation: Subjects reported to the Exercise Laboratory about 2 h prior to the start of the exposure. Body weight was recorded after the subjects urinated. All subjects drank 5 ml/kg of water to ensure uniform hydration. A venous blood sample was obtained from a forearm vein by aseptic venipuncture.

A thermistor was inserted 15 cm beyond the anal sphincter to measure core temperature. Heat flux transducers with imbedded thermistors were placed on 8 skin sites: forearm, triceps, chest, upper back, abdomen, anterior thigh, posterior thigh, and calf.

Three ECG electrodes were placed on the sternum in order to obtain heart rate. An external urinary catheter (Hollister Inc., Libertyville, IL) was attached to the penis and connected to a urine collection bag. This arrangement permitted the subjects to urinate ad libitum without having to exit the water.

5. MEASUREMENTS

a. Thermal Balance: Regional skin temperatures and heat fluxes, plus rectal temperature, were recorded each minute in the dry and wet phases. Mean skin

temperature and total body heat flux were obtained from the sum of regional temperatures and fluxes, weighted for body surface area. Thermal balance was estimated from the difference between metabolic heat production and heat loss. Heat production was calculated from oxygen consumption and the respiratory exchange ratio. Heat loss was represented by total body heat flux.

b. Perceived Thermal Sensation: Subjective evaluation of perceived thermal sensation was obtained every 30 min during the dry phase, and immediately after completing each exercise period during the wet phase. The 0-8 scale of Gagge[56] was used to score thermal sensation; where 0 is "very cold" and 8 is "very hot".

c. Respiratory Variables: During the dry phase, oxygen consumption ($\dot{V}_{O_2}$) and minute ventilation ($\dot{V}_E$) were obtained from timed 5-minute volume collections of exhaled gas. Collections were made every 30 min during the dry phase. Exhalations were collected in weather balloons and emptied through a calibrated dry gasometer to obtain $\dot{V}_E$. Oxygen and carbon dioxide concentrations were sampled on the outflow side of the gasometer, and analyzed with infrared (Model 540, Sybron Taylor, Rochester, NY) and paramagnetic (Model LB-2, Sensor Medics, Anaheim, CA) instruments, respectively.

During the wet phase respiratory exhalations were measured by a heated pneumotachograph (Model 4813, Hans Rudolph, Kansas City, MO). Exhaust gas was directed through respiratory tubing from the exhaust side of the demand regulator to the pneumotachograph. A check valve on the exhaust side of the regulator prevented free flow of gas due to the difference in hydrostatic pressure from the mouth to the surface of the water. The pneumotachograph was calibrated at 20 fsw using a respiratory syringe to

move a known volume of gas through the sensor at different flow rates. Output signals from the pressure transducer attached to the pneumotachograph were digitized and stored on a computer. Computer software was used to convert gas flow to minute volume. Ventilation was sampled for 2-minute periods every 10 min during the first 2 h and at the end of each moderate workload during the last hour. During sampling periods the exhaled gas was collected in weather balloons on the downstream side of the pneumotachograph in order to measure O_2 and CO_2 concentrations.

d. Strength and Dynamic Exercise: Handgrip strength was measured during the 1st and 4th hours of the dry phase using a handgrip dynamometer. The dynamometer was calibrated by suspending known weights (20-60 kg) from the handle and recording the output reading on the dynamometer dial. Maximum grip strength of the right arm was determined from 3 trials at the start of the dry phase. The decline in grip strength during a 1-minute sustained isometric contraction, beginning at 80% of maximal grip strength, was used to quantify changes in grip force.

Changes in heart rate, respiratory variables, and perceived exertion during the wet phase were used to quantify differences between drug vs placebo and between air vs O_2 breathing. Since all divers were able to complete the assigned workloads, endurance at these levels of exertion were not affected by test conditions. However, the physiological changes associated with each experimental condition provided an estimate of the magnitude of physical stress imposed during the dives.

e. Relative Perceived Exertion: Subjective evaluation of perceived exertion during the immersed exercise periods was accomplished by using the 0-10 Borg scale,[55] where 0

is perceived as "very easy" and 10 is perceived as "extremely hard". Evaluations were obtained immediately after cessation of each exercise period during the wet phase.

f. Cognitive Performance: The NMRI Performance Assessment Battery (PAB) test was used to assess cognitive changes during each exposure. The PAB test consisted of two cognitive tasks, Matching-To-Sample and Repeated Acquisition. The former task measures short-term working memory and recognition of spatial patterns, while the latter task measures short-term memory and on-site learning capability. Tests were administered via a computer keyboard display, with each PAB test requiring 20-30 min to complete. Prior to the first exposure subjects completed 10-12 PAB tests in order to achieve a plateau on the learning curve, with the tests administered under thermoneutral conditions. On exposure days the PAB tests were given approximately 30 min prior to the start of the exposure, after 2 h into the dry phase, at the end of the 4th hour of the dry phase, and 20-30 min after completing the wet phase. Computer terminals were placed in the dry chamber to administer PAB tests during the exposures.

g. Mechanical Coordination: This test required the subject to assemble 12 pairs of washers and nuts onto a 4-inch bolt as quickly as possible. The time to complete the task was taken as the measure of overall coordination. Tests were conducted during the 1st and 3rd hours of the dry phase, and during the 1st and 3rd 10-minute rest periods of the wet phase (first 2 h of immersion). During the immersed testing the tray containing the washers, nuts, and bolt was situated underwater between the subjects.

h. Visual Acuity: Plastic cards with black lettering on white background were used to assess visual acuity. A series of 8 letters and numbers were imprinted on each of 7

cards. The card held 3 feet from the subject, plus the size of the lettering, required a minimal visual acuity of 20/40 to accurately read the card. Vision tests were conducted during the 1st and 3rd hours of the dry phase, and during the 2nd 10-minute rest period during immersion. At the start of each test, the chamber ports were covered and all lights except one were turned off to control background lighting. Each test consisted of two parts. First, a card was held 3 feet from the subject while ambient chamber lighting was reduced to the point where the subject could no longer discern the lettering clearly. The rheostat setting of the light was recorded as the acuity point of going from light to dark. The second part of the test began by switching to another card and turning the rheostat setting to zero. The rheostat setting was then gradually increased until the subject could correctly identify the lettering on the card. The rheostat setting at this point was used to quantify acuity going from dark to light conditions.

i. Hydration Status and Blood Variables: Changes in body weight were measured as the difference in weight between pre-exposure and post-exposure. This served as a reliable index of net loss of body fluid. Urine volumes were collected during the dry and wet phases to assess the net fluid intake-output. One liter of water per hour during the dry phase constituted the intake. Total urine production (dry + wet) was used as fluid output. It was technically not possible to measure insensible water loss.

Venous blood samples were obtained by venipuncture of a forearm vein prior to the start of the exposure, at the end of the dry phase, and within 20 min of completing the wet phase. Samples were analyzed for hemoglobin, hematocrit, glucose, and lactate. Changes in blood and plasma volumes were derived from height, weight, hematocrit, and

hemoglobin. A portion of each sample was frozen for later analysis of serum cholinesterase activity.

j. Drug Side Effects/Oxygen Toxicity: Six questions were asked of subjects immediately following each exercise period in the wet phase. The questions and responses were to determine whether subjective measures of neuromuscular dysfunction were present that might indicate a side effect of pyridostigmine or a sign of oxygen toxicity. Content of the questions related to: feeling nauseated, visual disturbances, muscle twitching or cramps, tinnitus (ringing in ears), headache, and general overall feeling.

6. GENERAL RESULTS

a. Abort Criteria and Completed Exposures: An upper limit of 39.5 ° C in rectal temperature was used as an abort criteria for thermal stress. In addition, since heart rate is known to rapidly and suddenly increase when thermoregulatory ability fails, a heart rate equal to 90% of the subject's maximal value was also used as a thermal abort endpoint. No subject had a rectal temperature above 39 ° C, nor did any subject approach their heart rate limit. Thus, no exposures were aborted because of thermal stress.

Two exposures were aborted during the wet phase, once with a subject breathing air and once with a subject breathing oxygen. In both cases the subjects experienced difficulty breathing through the demand regulator of the full face mask. These

difficulties arose within the first hour of immersed exercise and produced respiratory discomfort of a sufficient degree to warrant termination of the exposures.

Table 3 presents the number of subjects tested under each condition. The first test exposure involved the two supplemental divers breathing air, in the absence of any drug. One diver was aborted during the wet phase for reasons mentioned above. The other diver successfully completed the dive, but appeared to be working quite hard, although his level of physical fitness was quite high. In addition, he developed orthostatic intolerance after exiting the water.

The next 10 exposures involved the 10 test subjects ingesting either drug or placebo, and breathing 100% oxygen at 20 fsw. Except for the one subject abort (5th exposure, placebo) for regulator malfunction, all the subjects completed the wet phase without difficulty. None appeared near exhaustion during the work cycles, and there were no incidences of post-immersion orthostatic intolerance. This raised the question as to whether there was a notable difference on work tolerance between breathing air vs 100% O_2. Subsequently, 5 additional exposures were conducted, each in the absence of drug.

The first of the additional exposures involved testing the 2 supplemental divers breathing 100% O_2 instead of air. Both divers completed this test without difficulty and did they exhibit any evidence of post-immersion orthostatic intolerance. Eight of the experimental subjects were tested on the next 4 exposures, breathing air during the wet phase. None of these subjects experienced any difficulty in completing the immersed work, nor did they exhibit any signs or symptoms of post-immersion orthostatic intolerance. Subjects 9 and 10 were not tested because one developed an unrelated

upper respiratory infection, and the other subject was the one aborted during the first trials. No explanation can be offered as to why the supplemental diver had such a hard time during his first exposure (on air).

b. Pyridostigmine Results: Table 4 presents the general findings of pyridostigmine effects on thermal, exercise, and cognitive aspects of this study. None of the findings were considered to represent an effect of the drug that would compromise a diving mission. Cognitive performance was unaltered by pyridostigmine. Mean skin temperature and heart rate during the dry phase were the only variables that exhibited a statistically significant effect by pretreatment with the drug. The 0.1-0.2 ° C reduction in skin temperature in the drug trial was, however, insufficient to alter body heat stores when compared to placebo trials. A 7 ± 2 beats/min reduction in resting heart rate occurred after pretreatment with drug. No significant differences between drug and placebo were noted during the wet phase for any measured variable.

c. Thermal Stress Results: Table 5 summarizes those findings that can be ascribed to heat stress alone. Values measured during the placebo trials, breathing 100% O_2 in the wet phase, were used to construct the table. Core temperature increased by only 0.2 ° C during the dry phase, increased 0.4 ° C upon immersion in 34.4 ° C water, remained reasonably constant during light exercise, and increased 0.3 ° C during the last hour, which involved moderate exercise. The final average rectal temperature of 38.0 ± 0.1 ° C represented a 0.9 ° C increase over the 7-hour period of exposure.

Oxygen consumption ($\dot{V}_{O_2}$), minute ventilation ($\dot{V}_E$), and heart rate (HR) were comparable to values expected for similar workloads conducted in cooler water.

The average loss of body weight was 1.3 ± 0.2 kg, which did not correlate with the difference between fluid intake minus urine volume. This finding may be explained by loss of body fluid through sweating or other avenues not measured in this study. Urine produced during the dry phase (2204 ± 203 ml) was greater than would occur normally in subjects not drinking any fluid (~480 ml). Urine volume during immersion (1270 ± 197 ml) was slightly greater than noted for subjects resting in 35 ° C water (920 ± 150 ml) for 3 h.[58]

d. Air vs O_2 Diving: Table 6 summarizes the difference between breathing air vs 100% oxygen during the wet phase. These results were compiled from air trials and from O_2 trials in the absence of pyridostigmine. Heart rate was significantly higher by 11 ± 4 beats/min at the moderate workload when breathing air than when breathing O_2. The slightly lower $\dot{V}_E$ at 25 W breathing air was not significantly different than values obtained breathing 100% O_2. All other values of $\dot{V}_E$ and $\dot{V}_{O_2}$ were the same between air and O_2 conditions.

7. SUMMARY

(a) Pretreatment with pyridostigmine (30 mg every 8 h) presents no limitation to divers performing light to moderate work in 94 ° F water at 20 fsw.

(b) No signs or symptoms of acute oxygen toxicity were noted at 20 fsw. The drug did not appear to alter the risk of oxygen toxicity at this depth.

(c) Heat stress alone reduced cognitive performance 20-40% in tasks involving short-term memory, spatial recognition, and on-site learning.

(d) Core temperature reached an average value of 38.0 ° C after 3 h in 94 ° F water, a value not considered to represent a severe hyperthermic stress.

(e) Breathing air vs 100% oxygen at 20 fsw did not significantly affect physical performance at light-moderate rates of work.

(f) Pre-dive fluid ingestion (1 ℓ/h) is a necessary prerequisite to avoid major problems with dehydration associated with diving.

8. LAY LANGUAGE SUMMARY:

Pyridostigmine is a drug that can be used as pretreatment prophylaxis against potential exposure to chemical nerve warfare agents. This study was done to determine whether pretreatment with this drug would alter a diver's ability to perform work in warm water.

Ten U.S. Navy divers participated in two dive tests, once after ingesting pyridostigmine (30 mg every 8 h) for two days and once after taking a placebo (no drug). Each test dive consisted of a 4-hour pre-dive period at the surface where air temperature was 100 ° F, followed by a 3-hour dive to 20 feet seawater where water temperature was 94 ° F. In-water exercise was conducted at light to moderate rates of work that were similar to swimming with fins at a speed of 0.6-1.1 knots. The divers breathed 100% oxygen at depth. During the pre-dive dry phase, pretreatment with pyridostigmine resulted in a slightly lower skin temperature, but this was not great enough to produce changes in body heat stores. Heart rate was 7 beats per minute lower with the drug.

No significant effects of pyridostigmine were noted during the dive. Thermal balance, heart rate, oxygen consumption, and respiratory ventilation values were similar to tests conducted without the drug. There were no signs or symptoms of acute oxygen toxicity or drug side effects, indicating no interaction between pyridostigmine and hyperbaric oxygen.

The magnitude of the environmental heat stress used in this study produced a core temperature at the end of the dive of 38.0 ° C (normal = 37.0 ° C). Since core temperatures above 39.0 ° C are considered to represent hyperthermia, a value of 38.0 ° C is well below the point where dive missions might be limited by heat stress. On the other hand, cognitive function was reduced 20-40% during these heat exposures. This finding suggests that the ability to retain short-term memory, recognize spatially oriented objects, or learn new tasks on-site is compromised; which in turn, may limit a diver's mission effectiveness.

Based on oxygen consumption, a volume of 6.5 ft^3 of oxygen was used during the dive profile in this study. This would indicate that the duration of closed-circuit oxygen or mixed-gas underwater breathing apparatuses should not be a limiting feature for this type of 3-hour working dive. About 212 ft^3 of air would be required to complete the 3-hour dive profile, based on respiratory minute volume measurements. This would suggest that the duration of open-circuit scuba, using one set of double 80 ft^3 tanks, would be insufficient to complete a 3-hour dive using this protocol's work/rest paradigm.

Ingesting water, at the rate of one liter per hour, prior to warm water diving appeared to be an essential component of lessening the risk of dehydration or thermal

stress. The net decrease in body weight after these 7-hour exposures was only 1.3 kg (3 lbs). It is likely that the divers, had they not ingested adequate fluid before the dive, would have had a greater loss in body weight indicative of marked dehydration. Since hydration status and tolerance to heat exposure are linked tightly, the need for adequate pre-dive hydration should be obvious.

In conclusion, the present study demonstrated that pretreatment with pyridostigmine produced no effects that would limit a diver's ability to work in warm water for periods up to 3 h. If the diver is adequately hydrated prior to the dive, then performing light-moderate work for 3 h in 94 ° F water poses no serious thermal stress. However, this level of heat exposure will reduce cognitive function; although whether this will limit missions depends, in part, on the type of mental tasks to be performed. Breathing air instead of 100% oxygen at 20 fsw does not appreciably influence overall physical performance.

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TABLE I: NMRI TECHNICAL REPORTS FOR THIS STUDY

Report #	Pyridostigmine and Warm Water Diving Protocol 90-05: TITLE	Authors	Topics
90-95	I. PROTOCOL AND GENERAL RESULTS	T.J. Doubt A.J. Dutka	Review of pyridostigmine Experimental overview General findings
90-96	II. THERMAL BALANCE	D.E. Hyde R.P. Weinberg D. Stevens T.J. Doubt	Core temperature Skin temperature Regional heat flux Body heat stores Perceived thermal sensation
90-97	III. COGNITIVE PERFORMANCE ASSESSMENT	J.R. Thomas J. Schrot S.T. Ahlers M.O. Thornton A.J. Dutka D.W. Armstrong K.R. Kowalski D. Shurtleff	Short-term memory Spatial recognition On-site learning Visual acuity Motor coordination
90-98	IV. PHYSICAL PERFORMANCE	T.J. Doubt J.R. Roberts N.A.S. Taylor R.P. Weinberg N.E. Holmes	Handgrip strength Heart rate Minute ventilation Oxygen consumption Perceived exertion
90-99	V. HYDRATION STATUS	D. Stevens D.E. Hyde J.W. Thorp K. Haberman T.J. Doubt	Body weight changes Fluid intake Urine production Plasma volume Venous glucose and lactate Orthostatic tolerance

TABLE 2: TEST SUBJECT PHYSICAL CHARACTERISTICS

SUBJECT	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	MEAN SKIN FOLD (mm)	BODY FAT (%)	MAX V_E (ℓ/min)	MAX V_{O_2} (ml/kg/min)	MAX HR (bpm)
1	33	182	74.46	6.8	13	129	39	200
2	31	184	92.20	6.8	15	189	50	201
3	27	172	64.86	7.5	14	179	48	193
4	28	183	79.14	6.5	12	139	42	185
5	28	170	70.12	7.1	15	144	45	188
6	37	177	80.34	7.6	20	147	40	178
7	25	182	74.36	6.7	11	142	50	199
8	33	183	94.22	9.7	21	153	35	187
9	29	183	86.40	6.3	12	147	43	178
10	29	178	76.68	5.9	15	168	51	186
MEAN =	30	179	79.28	7.1	15	154	44	190
S.D. =	4	5	9.36	1.1	3	19	5	9

NOTE: 1) Mean skinfold thickness determined from 11 sites: biceps, triceps, subscapula, lower back, forehead, forearm, chest, abdomen, ant. thigh, post. thigh, calf.

2) % Body fat measured by bioelectric impedance.

3) Maximum ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), and heart rate (HR) measured during incremental cycle ergometer testing in dry lab.

TABLE 3: TESTING SEQUENCE AND ABORTED TRIALS

EXPOSURE	SUBJECTS (DRUG CODE)	DIVE GAS	ABORTED
1	A (O), B (O)	AIR	A
2	1 (DR), 2 (PL)	100% O_2	--
3	3 (PL), 4 (DR)	100% O_2	--
4	5 (DR), 6 (PL)	100% O_2	--
5	7 (PL), 8 (DR)	100% O_2	--
6	9 (DR), 10 (PL)	100% O_2	10
7	1 (PL), 2 (DR)	100% O_2	--
8	3 (DR), 4 (PL)	100% O_2	--
9	5 (PL), 6 (DR)	100% O_2	--
10	7 (DR), 8 (PL)	100% O_2	--
11	9 (PL), 10 (DR)	100% O_2	--
12	A (O), B, (O)	100% O_2	--
13	1 (O), 2 (O)	AIR	--
14	3 (O), 4 (O)	AIR	--
15	5 (O), 6 (O)	AIR	--
16	7 (O), 8 (O)	AIR	--

DRUG CODES: 0 = NO DRUG, DR = PYRIDOSTIGMINE, PL = PLACEBO

Total Drug tests completed: n = 10 subjects
Total Placebo tests completed: n = 9 subjects
Total Air tests completed: n = 9 subjects

Subjects A, B were supplemental divers; no DR/PL trials

TABLE 4: FINDINGS PYRIDOSTIGMINE VS PLACEBO

AREA	MEASUREMENT	EFFECT OF DRUG
THERMAL	skin temp.	0.1-0.2 ° C lower, dry phase
	core temp.	none
	thermal sensation	none
	resting heart rate	7 beats/min lower, dry phase
COGNITIVE	PAB	none
	Coordination	none
	Visual acuity	none
EXERCISE	Handgrip strength	none
	In-water exercise	none
	Perceived exertion	none
HYDRATION	Pre-post loss weight	none
	Urine production	none
	Hemoglobin/hematocrit	none
	Glucose/lactate	none
DRUG SIDE EFFECTS OBSERVED		none
INCIDENCE OXYGEN TOXICITY		none

TABLE 5: RESULTS OF HEAT STRESS ALONE
(Placebo trials, O_2 breathing)

AREA		RESULTS
THERMAL	skin temp.	2 ° C increase in dry peak in wet = 35.2 ° C
	core temp.	0.2 increase in dry peak in wet = 38.0 ° C
COGNITIVE	PAB	20-40% decrease
	Coordination	unrelated to time/core temp
	Visual acuity	unrelated to time/core temp
EXERCISE	Grip strength	no decrease with time in dry
	In-water exercise	$\dot{V}_{O_2}$: light = 1.06 ± 0.06 ℓ/min mod. = 1.87 ± 0.38 ℓ/min $\dot{V}_E$: light = 36 ± 1 ℓ/min mod. = 51 ± 8 ℓ/min HR: light = 111 ± 10 bpm mod. = 136 ± 11 bpm
HYDRATION	Loss body weight	1.3 ± 0.2 kg
	Urine volume	dry: 2204 ± 203 ml wet: 1270 ± 197 ml

TABLE 6: COMPARISON AIR vs O_2 BREATHING

(34.4 ° C water, 20 fsw, no drug)
(mean ± SEM)

MEASUREMENT	WORK	AIR	OXYGEN	
Rectal temp. (° C)	25 W	37.6 ± 0.1	37.7 ± 0.1	ns
	1 W/kg	38.0 ± 0.1	38.0 ± 0.1	ns
Mean skin temp.(° C)	25 W	34.9 ± 0.1	35.0 ± 0.1	ns
	1 W/kg	35.0 ± 0.1	35.2 ± 0.1	ns
Heart rate (beats/min)	25 W	115 ± 11	112 ± 10	ns
	1 W/kg	145 ± 13	138 ± 11	$p<0.05$
Ventilation (ℓ/min)	25 W	32 ± 1	36 ± 1	ns
	1 W/kg	52 ± 4	52 ± 4	ns
Oxygen uptake (ℓ/min)	25 W	1.03 ± .04	1.06 ± .06	ns
	1 W/kg	1.83 ± .14	1.90 ± .14	ns

ns = no significant difference Air vs O_2